1. INTRODUCTION

Childbearing and family are considered a right of every human being. According to World Health Organisation (WHO) (2020), “Infertility is a serious health issue affecting millions of people of reproductive age worldwide. Between 48 million couples and 186 million individuals have infertility globally”. WHO (2020) also stated that, “infertility is a disease of the male or female reproductive system defined by the failure to achieve a pregnancy after 12 months or more of regular unprotected sexual intercourse”.

Poongothai et al (2009) estimated that, “in India around 15 to 20 million (25%) individuals were reported with infertility”. Adamson et al in 2011 reported that, “infertility affects millions of couple around the world and is still increasing”. Olive et al in 2014 explained that, “both female and male factors can lead to infertility”.

In 2015, Masoumi et al suggested that, “causes of infertility are numerous such as anatomical, physiological and genetic factors”. According to American Society for Reproductive Medicine (ASRM) (2008) it was explained that, “infertility is a multifactorial disease with etiologies attributed to male, female, and unexplained factor”. In previous study by Chemes (2000) suggested that, “even though many of the infertile couples suffered from idiopathic infertility, genetic factors also play a significant role in infertility”.

Masoumi et al (2015) reported that, “many environmental and acquired factors also influence fertility and may lead to infertility. Menstrual and ovulation dysfunction and uterine factors are the most common causes of impairment in fertility”. In a study byMacaluso et al (2010) denoted that, “etiology of infertility, prevalence and patterns of causes of infertility in different regions are diverse. This difference is due to existence of differences in environmental conditions associated with reproductive behaviors, such as age at marriage, environmental

pollution, habit of smoking and alcohol consumption, changing in lifestyle and dietary pattern".

Masoumi et al (2015) suggested that, "menstrual and ovulation disorders and uterine factors are among the most common causes of female infertility". According to Shokoohi et al (2018),"male infertility factors are known to reduce the production of sperms with normal morphology and progressive motility". Meng et al (2015) reported that, "although infertility affects human fertility health status and there is a great deal of concern about identifying its risk factors, there are no comprehensive epidemiological studies about infertility risk factors".

According to Smith et al (2003), "unexplained infertility refers to the 30% to 40% of infertile couples in whom standard investigations, including semen analysis and tests of ovulation and tubal patency, cannot detect any major abnormality". In a study done by Siristatidis and Bhattacharya (2007) it was mentioned that, "couples with unexplained infertility suffer from difficulties in fecundity. The possible underlying etiologies are diminished ovarian reserve, advanced age, minimal and mild endometriosis, endocrine abnormalities, oxidative stress and defective endometrial receptivity".

Bevc et al (2008) reported that, "Cardiovascular disease (CVD) is the one of the most common causes of morbidity and mortality in the world, and atherosclerosis is known to be the main reason for increased cardiovascular risk". Moreover, Bevc et al (2008) added on that, "it is widely known that inflammation plays an important role in atherosclerosis".Verit et al (2014) reported that, "CVD risk factors were found to be increased in patients with diminished ovarian reserve". Many studies (Purcell and Mole 2011; Michalakis and Coppack 2012) have shown that,"aging, obesity, dyslipidemia, and chronic inflammation are related with reduced oocyte quality and quantity".

Significance of the study

One in every four couples in developing countries is found to be affected with infertility. Both men and women with infertility have an increased risk for developing chronic conditions such as diabetes and heart disease. Since cardiovascular disease (CVD) is a leading cause of morbidity and mortality throughout the world, it is very important to have an awareness of the relation between infertility and cardiovascular diseases and its potential risk factors. Thus it can be helpful for the infertile individuals for the early diagnosis of disease and its management.

According to Valkenburg et al (2008), “the relationship between female factor infertility subtypes (such as ovulatory disorders, endometriosis, and uterine fibroids) and cardiovascular disease risk may be mediated by different pathways”. Zhang et al (2012) explained that, “women with PCOS have greater prevalence of elevated total cholesterol, triglycerides, LDLas well as lower HDL”.

Rizzo et al in 2009 reported that, “the pathway from infertility to CVD may be mediated by hyperandrogenism, dyslipidemia, obesity, and insulin resistance”. In 2017, Mahalingaiah et al denoted that,“aside from ovulatory disorders and endometriosis, the relationship between infertility and CVD risk factors is still unclear”. It was mentioned in study done by Farland et al (2015) that, “the association of infertility history, infertility treatment and pre-menopausal cardiovascular disease with no association".

However, due to the conflicting literature, there is still concern that couples suffering with infertility may be a susceptible population at risk for cardiovascular disease.

Need for the study

Infertility is a global health issue affecting approximately 8–15% of couples. An estimation of 60–168 million people worldwide suffering with infertility. Nowadays, cardiovascular diseases (CVDs) also became a leading

cause of mortality. It was estimated in several reports that, by the year 2030 more than 22.2 million people will die annually from CVDs. Growing evidence suggests that increased cardiovascular disease (CVD) risk is associated with female infertility caused by conditions such as polycystic ovarian disease, obesity, thyroid dysfunction and endometriosis. Women with subfertility have also increased levels of psychological stress, as manifested in conditions such as depression and anxiety, which may contribute to CVD.

Furthermore, the increasing prevalence of medical disorders such as diabetes, hypertension, and hypothyroidism and lifestyle diseases such as obesity and addictions in the young has also shown to contribute to the problem of infertility. Many studies have been undertaken worldwide and in India, to know the common causes of infertility. Moreover, infertility is not only a medical challenge but also takes a major cause in psychologically and financially on the couples suffering from infertility.

However, the conclusion of one or two studies is not quite enough to understand the pathophysiology of CVD risk factors among infertile subjects. It is because infertility itself a multifactorial problem as similar to cardiovascular diseases. Therefore, a proper study for understanding the relation between CVD and infertility and also the role of DNA repair and damage should be carried out to limit the future risk of having CVD conditions. Moreover, the role of oxidative stress and somatic DNA damages in infertility has not been studied extensively. Traditional risk factors and existing risk prediction models are very good predictors of CVD risk in both women and men, which leaves a comparatively small space for as yet unidentified biomarkers to emerge as important factors for risk stratification. Hence, the present study was carried out to evaluate the role of genetic, physiological and metabolic biomarkers for predicting CVD among infertile subjects.

2. Aim

The present study was undertaken to evaluate the genetic, physiological and metabolic biomarkers for predicting cardiovascular disease among infertile subjects.

Objectives

- To quantify the extent of somatic DNA damages in infertile subjects by Cytokinesis-block Micronuclei (CBMN) Assay.
- To assess the DNA repair proficiency of these infertile subjects by Mutagen induced chromosome sensitivity analysis.
- To evaluate the chromosomal abnormalities, if any, present in couples with infertility by Karyotyping.
- To assess the biochemical characteristics associated with infertility and its risk for cardiovascular disease, by evaluating the Total Cholesterol (TC), Blood Glucose and Triglyceride levels (TG).
- To assess, correlate and to predict the various genetic, physiological and demographic characteristics of infertile subjects and their risk for cardiovascular disease through the following investigations.

3. MATERIALS AND METHODS

In the present study 180 couples who were suffering from infertility were selected as study subjects. These subjects were referred from various infertility centres of Kerala, to Genetika, Centre for Advanced Genetic Studies, Trivandrum. Detailed demographic, anthropometric, physiological and lifestyle characteristics were recorded using proforma. One hundred and forty age and sex matched healthy couples were selected as controls. Age of the male study subjects ranged from 21-50 years and female subjects it was between 18 to 45 years. Neither the patients nor the controls were suffering from any acute or chronic illness, cancer or on prolonged medication for any other purpose and also

subjects above the age of 55 and below the age of 18 were excluded. Eight ml (8ml) of venous blood was collected after 10-12 hours of fasting. 3ml of blood was transferred aseptically to a sodium heparinsed vacuutainer for evaluating the DNA repair proficiency by mutagen induced chromosome sensitivity analysis, to quantify the extent of somatic DNA damages by Cytokinesis-Block Micronuclei (CBMN)assyand also for karyotype analyses. The rest of blood was transferred to a plain tube, serum separated after clot formation and investigations were done for biochemical test. The following investigations were performed. T3, T4, TSH, fasting blood sugar, serum triglyceride, serum total cholesterol, serum HDL, serum LDL, malondialdehyde, hsCRP, urea, fibrnogen and creatinine.

4. OBSERVATIONS AND RESULTS

Statistical analysis was performed using the Statistical Package for the Social Science (SPSS) software, version 16. Continuous variables were expressed in mean and standard deviation. In both categorical and continuous variables, Chi square and t test were used to compare the difference between study and control groups; p-value less than 0.05 were considered as statistically significant. ANOVA was carried out to find the correlation between hsCRP, fibrinogen, MDA, mean CBMN frequency and mean b/c value with categorical variables. In addition, Pearson correlation was used to find out the correlation between hsCRP, fibrinogen, MDA, mean CBMN frequency and mean b/c value with the continuous variables. To identify independent predictor, univariate data were converted to multivariate data.

The statistical analysis was performed using the Chi Square test for categorical variables in female subjects. Characteristics like physical activity, frequency of fruit consumption, water intake per day and menstrual periods showed a statistical significance (p value <0.05) between the study and control

group. The odds ratio of the associated factors was calculated and the risk of cardiovascular disease among the female subjects was observed.

The t-test was used to compare continuous variables between two groups. Parameters such as, BMI, duration of married life, FBS, total cholesterol, HDL-C, LDL-C, triglycerides, urea, creatinine, T3, T4, TSH, uric acid, MDA, hsCRP, fibrinogen, mean CBMN frequency and mean b/c value showed a statistical significant difference between the female study groups and control subjects.

Pearson correlation was done for assessing the correlation of MDA. It was observed that, hsCRP, fibrinogen, mean CBMN frequency, mean b/c value, FBS, total cholesterol, LDL-C, triglycerides, urea, TSH and uric acid showed a positive correlation, whereas, HDL-C, T3 and T4 showed a negative correlation with MDA. The Pearson correlation analyses of hsCRP with other parameters revealed that, characteristics such as, MDA, fibrinogen, mean CBMN frequency, mean b/c value, FBS, total cholesterol, LDL-C, triglycerides, urea, creatinine, TSH and uric acid showed a positive correlation, whereas, HDL-C, T3 and T4 showed a negative correlation with statistical significant difference.

Parameters such as, MDA, hsCRP, mean CBMN frequency, mean b/c value, FBS, total cholesterol, LDL-C, triglycerides, urea, TSH and uric acid showed a positive correlation with fibrinogen in Pearson correlation analysis. However, parameters like HDL-C, T3 and T4 showed a negative correlation with fibrinogen. A strong positive correlation was observed among parameters such as MDA, hsCRP, fibrinogen, mean b/c value, FBS, total cholesterol, LDL-C, triglycerides, urea, TSH and uric acid with mean CBMN frequency. However, certain parameters such as, HDL-C, T3 and T4 showed a negative correlation with mean CBMN frequency.

Pearson correlation was done for assessing the correlation of mean b/c value. It was observed that, MDA, hsCRP, fibrinogen, mean CBMN frequency, FBS, total cholesterol, LDL-C, triglycerides, urea, TSH and uric acid showed a

positive correlation, whereas, HDL-C, T3 and T4 showed a negative correlation with mean b/c value.

Parameters which showed a statistical significance in univariate analysis were subjected to multivariate analyses. Multiple regression models for MDA were performed. It was observed that, variables like irregular exercise, type of oil used, total cholesterol, HDL-C and uric acid were observed as the independent predictors of oxidative stress in infertile female subjects.

Multiple linear regression model of hsCRP revealed that, variables like irregular menstrual periods, FBS, HDL-C, triglycerides, creatinine, TSH and uric acid level were also observed as the independent predictors of inflammatory marker in female infertile subjects. In case of multiple linear regression model of fibrinogen, parameters such as, educational status, H/o hypertension, irregular exercise, total cholesterol, HDL-C, urea, TSH and uric acid observed as the independent predictors. Regression analysis of mean CBMN frequency showed that, irregular exercise, poor fruit consumption, menstrual periods, total cholesterol, HDL-C, urea, TSH and uric acid are independent predictors of DNA damages in infertile female subjects. Multiple linear regression model of mean b/c value showed that, characteristics such as irregular exercise, poor fruit consumption, total cholesterol, HDL-C, TSH and uric acid are independent predictors of DNA repair efficiency in female infertility.

In male study subjects t test was performed and it was observed that, anthropometric parameters (height, weight, abdominal circumferences and BMI), demographic factor (birth order and duration of married life), laboratory parameters such as, FBS, total cholesterol, HDL-C, LDL-C, triglyceride, urea, creatinine, T3, T4, TSH, uric Acid, MDA, hsCRP, fibrinogen, mean CBMN frequency and mean b/c value showed a statistically significant difference between the study and control subjects.

The statistical analysis of categorical variables was done by Chi square test. An observable statistically significant difference was noticed in parameters such as, family history of (FH/o) infertility/subinfertility, History of (H/o) hypertension, H/o dyslipdemia, FH/o CAD, regular exercise, physical activity, type of oil used, frequency of fruit consumption, water intake per day, obesity and semen analysis. Odds ratio of the categorical variables was also calculated for assessing the strength of association of these variables with infertility among male study subjects.

Relationship of MDA, hsCRP, fibrinogen, mean b/c value and mean CBMN frequency with continuous variables was analyzed by Pearson correlation. In case of MDA, variables like, hsCRP, fibrinogen, mean CBMN frequency, mean b/c value, FBS, total cholesterol, LDL-C, triglyceride, urea, creatinine, TSH, uric acid and birth order showed a strong positive correlation whereas, HDL-C and T3 showed a negative correlation.

Parameters such as, MDA, fibrinogen, mean CBMN frequency, mean b/c value, FBS, total cholesterol, LDL-C, triglyceride, urea, creatinine, TSH, uric acid and birth order showed a strong positive correlation with hsCRP among study and control groups. However, HDL-C, T3 and T4 showed a negative correlation with hsCRP.

Pearson correlation was done for assessing the correlation of fibrinogen. It was observed variables like, MDA, hsCRP, mean CBMN frequency, mean b/c value, FBS, total cholesterol, LDL-C, triglyceride, Urea, creatinine, TSH, Uric acid and birth order showed a strong positive correlation with fibrinogen among study and control groups. Whereas, HDL-C, T3 and T4 showed a negative correlation with fibrinogen in Pearson correlation.

The Pearson correlation analyses of mean CBMN frequency with other parameters were analyzed and it was observed that characteristics such as, MDA, hsCRP, fibrinogen, mean b/c value, FBS, total cholesterol, LDL-C, triglyceride,

urea, creatinine, TSH, uric acid and birth order showed a strong positive correlation. Other parameters like HDL-C, T3 and T4 showed a negative correlation with mean CBMN frequency.

Variables like MDA, hsCRP, fibrinogen, mean CBMN frequency, FBS, total cholesterol, LDL-C, triglyceride, urea, creatinine, TSH, uric acid and birth order showed a strong positive correlation with mean b/c value; whereas, HDL-C, T3 and T4 showed a negative correlation.

The results of multiple linear regressions for MDA revealed that, characteristics such as, H/o hypertension, irregular exercise, FBS, HDL-C, Triglyceride, urea and uric acid are independent predictors of oxidative stress in infertile males. In case of multiple linear regression model of hsCRP, parameters such as, irregular exercise, HDL-C, TG, urea, TSH and uric acid observed as the independent predictors of inflammatory marker in infertile male study subjects. Multiple linear regression model of fibrinogen showed that, total Cholesterol, HDL-C, urea and uric acid were observed as the independent predictors of inflammatory marker in infertile male study groups.

Regression analysis of mean CBMN frequency showed that, variables like H/o hypertension, irregular exercise, BMI, total Cholesterol, HDL-C, LDL-C, triglycerides, urea, TSH and uric acid are observed to be an independent predictors of DNA damage among infertile male study subjects. In the multiple linear regression model of mean b/c value showed that, parameters like H/o hypertension, irregular exercise, BMI, HDL-C, LDL-C, urea, TSH and uric acid as the independent predictors for DNA repair efficiency among male study group. Receiver operating characteristic curve (ROC) curve was also plotted to access the optimum cutoff value of predictors for CVD.

5. DISCUSSION

According to Mahalingaiah et al (2017), “infertility is a multifactorial disease with etiologies attributed to male, female and unexplained factors”. In the

present study 180 couples reported with infertility were selected as test subjects and the 140 healthy couples were selected as control subjects.In the present study, test subjects with infertility showed a decreased incidence of DNA repair efficiency and an increased DNA damage was observed in couples reported with infertility.

Katole and Saoji (2019) reported in study that, “out of 570 women of reproductive age group, 51 (8.9%) had primary infertility. Hence, the prevalence of primary infertility is 8.9% in women of reproductive age group (15–49 years) in urban population of Central India”. In the present study it was observed that, test subjects with advanced age showed an increased mean CBMN frequency and also an elevated men b/c value. Additionally, test subjects who reside in urban area showed increased mean CBMN frequency and mean b/c value. In another study Kumar (2007) investigated that, “extensive infertility problem in women aged 15–49 years from Khairwar to non Khairwar tribes in rural area of Central India”.

Mahalingaiah et al (2017) also mentioned that, “the main causes of female infertility are ovulatory disorders, including polycystic ovary syndrome (PCOS) and primary ovarian insufficiency (POI); endometriosis; hormonal and endocrine disorders, including thyroid and prolactin abnormalities; cervical mucus abnormalities; and structural or acquired genital tract disorders including uterine fibroids”.

In 2016, Anderson et al reported that, “women with ovulatory disorder infertility (PCOS and POI), some studies demonstrate increased prevalence and long term concern for cardiovascular disease risk”. In the current study, among female test subjects 14% (n=26) were reported with PCOS and observed mean b/c value was 0.844 and mean CBMN frequency was 13.67. It is clearly evident that an increased mean CBMN frequency and mean b/c value was observed among female test subjects with PCOS.

Chern et al (2012) suggested that, “age at menopause and menopausal status also alter the risk of cardiovascular morbidity and mortality”. In the current study out of 180 female test subjects, 16% (n=30) were reported with endometriosis. Moreover, an elevated mean b/c value and mean CBMN frequency was observed among these subjects.

Deshpande and Gupta(2019)reported that, “the causes of female infertility found, PCOS and tubal pathology were the most common causes contributing 46% and 33.8%, respectively, to all cases attributed to female infertility”. Study done by Mittal et al(2015)in Haryana,showed that,“the main female factor causing infertility is PCOS”.Jacob Farhi (2011)stated that, “studies done worldwide also prove that PCOS is the single most common cause of female factor of infertility”.

Moreover, Deshpande and Gupta (2019) also reported that, “around 20% of the causes of female infertility were attributed to uterine fibroids, endocrine abnormalities, or a combination of the above factors”.Among the comorbidities in infertile females, the most common ones seen are the endocrine abnormalities. In the current study, most of the female test subjects were reported with endocrine abnormalities.

In 2012, Verma et alwere found that, “the prevalence of hypothyroidism in infertile women to be 23.9%”.Deshpande and Gupta (2019) estimated that, “thyroid disorders were the most common endocrine abnormalities, seen in 21.6% of the infertile population who were screened for the same”. In the current study also several test subjects reported with thyroid disorder. An elevated mean CBMN frequency and mean b/c value was observed among test (couple) subjects compared to control group.

Mirzaei et al (2018) observed that,“there was a significant relationship between infertility and body mass index and waist circumference”. Dağ and Dilbaz (2015) suggested that, “obesity and overweight in women are rising and

obesity has harmful effects on body systems, especially fertility, and recent studies showed that the prevalence of obesity in infertile women is high and women with obesity and overweight are at risk of developing fertility diseases".In the current study, obesity was reported in 35% (n=63) of male study subjects and 23% (n=42) of female test subjects. Moreover, an increased mean CBMN frequency and mean b/c value was observed among test subjects reported with obesity.

According to Flynn (2006), "obesity, insulin resistance, and hypothyroidism are also related with infertility, anovulation, and CVD". In 2016, Kurabayashidemonstrated that,"women with ovarian infertility were at high risk of hypertension from 45 years of age, hypercholesterolemia at all ages, and diabetes mellitus before 45 years of age". Solomon et al (2002) reported, "a close relationship between menstrual irregularities and future CVD risk".

Parikh et al (2012) estimated that, "50% increased risk of myocardial infarction or coronary heart disease was found among women with menstrual irregularities in comparison with those who had normal cycles from the ages of 20 to 35 years". In the present study, 30% (n=55) female test subjects were reported with irregular menstrual cycle and their observed mean CBMN frequency and mean b/c value was 13.64 and 0.852 respectively.

Verit et al (2008) explained that, "advanced maternal age, minimal to mild endometriosis, endocrine abnormalities, diminished ovarian reserve, and oxidative stress have been identified as possible etiological factors for unexplained infertility".Moreover, Verit et al (2008) also stated that, "it is widely known that CVD increases with age. Endometriosis, another common cause of unexplained infertility, is characterized by local and systemic chronic inflammation, dyslipidemia and oxidative stress".In the current study also an increased MDA concentration was observed among test subjects when compared to the control groups. Moreover, a statistical significant difference was observed

among test and control group a statistical significant difference was observed with a p value <0.05.

Sotiriadis et al (2007) suggested that, “early miscarriages, which may be an etiological factor of childlessness, can result from coagulation disorders or thrombophilia”. Della-Morte et al (2012) observed that, “hypercoagulable states are associated with CVD risk. Furthermore, coagulation defects may affect the development of subclinical atherosclerosis and plaque formation”. In the current study, a statistically significant difference was observed in the TSH concentration among the test and control group.

In 2011, Shamila and Sasikala reported that, “socioeconomic status is one of the risk factors for infertility. In addition to, that the prevalence of primary infertility increases by aging, higher BMI, irregular menstrual pattern, and family history of infertility”. Das et al (2015) noted that, “as the changing trends of society, the level of education are increasing and also priorities of life are changing which prolongs the age at marriage”. In the present study based on the level of education there was no statistical significant difference was observed between test and control groups. Similarly, socioeconomic status among test and control.

In a study by Katole and Saoji (2019)reported that, “women having family history of infertility are at higher chances developing infertility problems mainly due to inherent genetic diseases. In addition, menstrual hygiene plays an important role in primary infertility”. In the current study, 11% (n=20) of female test subjects were reported with family history of infertility.

Agarwal et al in 2012 explained that, “strong evidence suggests that CVD is linked with oxidative stress. Oxidative stress also plays an important role in the etiology of unexplained infertility. Reactive oxygen species can negatively affect ovulation, fertilization and embryo quality”.In the present study it was observed

that, MDA concentration showed a statistically significant difference between the test and control group ($p<0.05$).

According to the study by Pacchiarotti et al (2007) it was reported that, “pregnancy and implantation rates have been found to be lower and abortion rates to be higher in women with elevated oxidative stress”. In the current study female test subjects showed an increased MDA concentration when compared to the control group. Moreover, test female subjects with MDA level <2.5µmol/L showed an elevated mean CBMN frequency (13.55) and mean b/c value (0.836).

In 2015, Hosen et al explained in a study that,“oxidative stress is an important factor which influences fertility potential and elevated seminal Reactive Oxygen Species (ROS) can cause sperm dysfunction through lipid peroxidation of the sperm membrane”.In the present study, 15% (n=27) test male subjects were reported with oligospermia, 9.5% (n=17) with azthenozoospermia, 10.5% (n=19) with azoospermia and remaining 65% (n=117) with normal sperm count and morphology.

In previous study by Agarwal and Allamaneni (2004) reported that, “small physiological levels of ROS are essential for normal sperm functions, i.e. sperm capacitation, the acrosome reaction and sperm-oocyte fusion, oxidative stress has been shown to be an important cause of male infertility”. In the current study the observed MDA concentration among male test subjects was, higher when compared to the control subjects. Inaddition to that, these test subjects with MDA concentration >2.5µmol/L showed an increased mean CBMN frequency (13.21) and mean b/c value (0.822) than the test group with MDA ≤2.5µmol/L.

Zarghami et al (2005) found that, “over production of ROS can cause DNA strand breaks, DNA cross-links and chromosomal rearrangements”.Shen and Ong (2000) observed that, “sperm DNA damage is closely related to male infertility and 8-OHdG is a sensitive marker of oxidative DNA damage”. In the current study it was observed that, test male subjects reported with

oligospermiashowed an increased mean CBMN frequency and mean b/c value when compared to rest.

Hsieh et al (2006) showed that, “lipid peroxidation impacts the sperm concentration, motility and morphology were associated with poor sperm quality”. Asbagh et al (2010) reported that,“there was a significant association between semen MDA and abnormal sperm morphology”. In the present study also MDA value increased with increasing mean CBMN frequency. The MDA value was more in infertile men than fertile men. Sharma et al in 2012 observed that,“heavy smoking resulted in reduced sperm number and alcohol consumption is associated with an increase in morphologically abnormal sperms”.

Studies done by Sharma et al(2016) andDeshpande and Gupta(2019) confirmed that,“both tobacco and alcohol being the most prevalent addictions significantly affecting semen quality”. In the current study, 17.7% (n=32) test (male) subjects were reported with habit of smoking and 22.7% (n=41) were reported with habit of alcohol consumption. In addition to that, both these subjects showed an elevated mean CBMN frequency and mean b/c value.

Parikh et al (2012) explained that, “it is widely known that infertility has been suggested to be a risk factor for CVD”. Wellons in 2011, found that, “CVD risk factors were found to be increased in patients with diminished ovarian reserve”. Purcell and Moley (2011) observed that,“aging, obesity, dyslipidemia and chronic inflammation are related with reduced oocyte quality and quantity”.

In 2009, Hansson reported that, “Insulin resistance and chronic low-grade inflammation are associated with cardiovascular risk, and these parameters can be used as CVD markers, as shown by many studies”. According to Rizzo et al (2009), “both of those markers induce many changes in blood lipid levels, leading to a proatherogenic lipid profile”. Verit et al (2017) estimated that, “TG, hsCRP, and HDL were independent variables correlated with unexplained infertility. The

identification of these parameters as independent variables strengthens the hypothesis of our study".

In the present study observed that, the incidence of various risk factors such as dyslipidaemia, diabetes and hypertension are more among test subjects. While in other studies done by Gardner et al (2000) and Rouvre et al (2011) reported that, "HDL-C improved with aging, there was a significant increase in total C, LDL-C and non-HDL-C. These changes in serum lipids are not specific to PCOS and also are observed in the general population".

In the current study it was also observed that, test subjects showed an increased TC, TG and LDL-C concentration and lower HDL-C level when compared to the control group.Carmina et al (2013)estimated that, "the increased serum LDL-C levels were observed in 26% of women in their 40s having an initial diagnosis of PCOS, and coupled with a similar increase in non-HDL-C suggest an increase in CV risk with aging".Verit et al (2017) observed,"a positive relationships were found between unexplained infertility and hs-CRP, TG, TC, and LDL, and a negative relationship was found with HDL. Of these markers, TG, HDL, and hs-CRP were independent predictors associated with unexplained infertility".

Kayed et al in (2006) reported that, "overall, 96.94% of the patients studied (5138/5300) had a normal karyotype, while the remaining 162 patients (3.06%) had an abnormal karyotype. Male patients constituted the majority of abnormalities- 138 males (85.19%) and 24 females (14.81%)". In the current study, out of 180 couples, 23 males (12.8%) and 15 females (8.3%) were reported with abnormal karyotype i.e. male patients constituted the majority of abnormalities.

Kayed et al in (2006) also estimated that,"chromosomal aberrations included 117 cases (2.2%) of sex chromosome abnormalities; 113 males and four

females. Forty-five patients (0.85%) had autosomal aberrations; 25 of them were males and 20 were females".

According to Shi and Martin (2000), "increases in chromosomal aberrations have been clearly demonstrated to increase proportionally with increasing severity of the infertility". In a study by Pylyp et al (2015) reported that, "chromosomal abnormalities were detected in 2.37% of cases (81/3414), corresponding to an abnormality frequency of 2.79% among men and 1.95% among women".

In the study done by Morris et al (2008) observed that, "numerical sex chromosomal abnormalities in males are relatively common with Klinefelter syndrome (47,XXY) and 47,XYY syndrome each occurring in approximately 1–2 in 1000 live births". In the present study, 5% (n=9) male test subjects were reported with numerical abnormalities and none of the female test subjects were reported with numerical abnormalities.

Hartonand Tempest (2012) reported that, "a high proportion of Klinefelter syndrome males are only identified when they undergo fertility assessments. The non-mosaic form of Klinefelter syndrome accounts for around 11% of azoospermic individuals, whereas mosaic individuals often present with oligozoospermia".Harton and Tempest(2012) reported in a study that, "chromosomal aberrations, either numerical or structural in nature, can have profound effects on fertility. The frequency of chromosomal aberrations in the general population is approximately 0.6%".

Gonçalves et al (2014) conducted a study in 240 abortion patients in Brazil and the observed,"prevalence of chromosomal abnormalities was 7.3% in women, the most abnormality associated with X chromosome mosaic and the frequency of chromosomal abnormalities in males was 2.1%". In the present study, test subjects with abnormal karyotype also showed an elevated mean b/c value when compared to control.

6. SUMMARY AND CONCLUSIONS

The study was conducted to evaluate the association of CVD among infertile subjects by performing genetic, physiological and metabolic biomarkers. And also an attempt was made to correlate the CVD risk factors with karyotype analysis using lymphocyte culture, somatic DNA damage using Cytokinesis-Block Micronuclei assay and DNA repair efficiency by using Mutagen Induced Sensitivity Analysis. Present study consist of 180 clinically diagnosed infertile couples as test subjects and 140 age and sex matched healthy couples as control groups. There are several factors that which may contribute to infertility, such as environmental factors, physiological factors, immunological factors and so on. In the present study, it was observed that, oxidative stress, somatic DNA damages, inefficient DNA repair mechanism and abnormal karyotype were also play an important role in female infertility.

The distribution of mean CBMN frequency according to demographic, clinical and biochemical characteristics of the study subjects was observed. An increased level of mean CBMN frequency was observed among test subjects with elevated level of FBS, total cholesterol, TSH, LDL-C, creatinine, hsCRP, MDA and fibrinogen. Similarly, an increased mean b/c value was observed among study subjects with elevated value of biochemical and immunological factors.

Statistical analysis was performed using the Statistical Package for the Social Science (SPSS) software, version 16. Continuous variables were expressed in mean and standard deviation. In both categorical and continuous variables, Chi square and t test were used to compare the difference between study and control groups. Multiple linear regression of hsCRP, MDA, fibrinogen, mean CBMN frequency and mean b/c value was analyzed among test and control group. Characteristics such as, hsCRP, MDA, fibrinogen, mean CBMN frequency and mean b/c value showed an increased incidence in test subjects when compared to the control groups. Even biochemical parameters and endocrinological

parameters showed a statistically significant difference between the test and control group with a p value <0.05.

Infertility is a major health problem that requires an appropriate treatment strategy. Modern medical science has developed advanced therapies to assist reproduction over the last 20 years. The main causes of infertility are lifestyle factors, environmental parameters, physiological abnormalities, genetic factors and other unknown causes. The diagnosis of chromosomal anomalies at the exact time can lead to the prevention of future birth of affected baby and hence the incidence of chromosomal abnormalities can be reduced to a certain extent in the society.

The findings of current study point out that, individuals suffering from infertility showed an increased incidence of risk factors which leads to CVD. Increased oxidative stress, decreased DNA repair efficiency and increased DNA damages are observed in infertile patients. Even test subjects with abnormal karyotype also showed an increased mean CBMN frequency, MDA concentration and mean b/c value. Therefore, it is better to prevent the increase of oxidative stress and DNA damages which leads to cardiovascular diseases. Healthy lifestyle factors, including exercise, are associated significantly with reduced DNA damage. Thus it can be concluded that certain dietary modification, changes in life style characters and proper medication can reduce the risk of CVD events and also other metabolic disorders related to infertility.

7. REFERENCE

1. https://www.who.int/health-topics/infertility#tab=tab_1 WHO 2020.
2. Macaluso, M., Wright-Schnapp, T.J., Chandra, A., Johnson, R., Satterwhite, C.L., Pulver, A., Berman, S.M., Wang, R.Y., Farr, S.L. and Pollack, L.A., 2010. A public health focus on infertility prevention, detection, and management. Fertility and sterility, 93(1), pp.16-e1.
3. Mahalingaiah, S., Sun, F., Cheng, J.J., Chow, E.T., Lunetta, K.L. and Murabito, J.M., 2017. Cardiovascular risk factors among women with self-reported infertility. Fertility research and practice, 3(1), pp.1-7.

4. Masoumi, S.Z., Parsa, P., Darvish, N., Mokhtari, S., Yavangi, M. and Roshanaei, G., 2015. An epidemiologic survey on the causes of infertility in patients referred to infertility center in Fatemieh Hospital in Hamadan. Iranian journal of reproductive medicine, 13(8), p.513.
5. Meng, Q., Ren, A., Zhang, L., Liu, J., Li, Z., Yang, Y., Li, R. and Ma, L., 2015. Incidence of infertility and risk factors of impaired fecundity among newly married couples in a Chinese population. Reproductive biomedicine online, 30(1), pp.92-100.\
6. Michalakis, K. and Coppack, S.W., 2012. Primary ovarian insufficiency: relation to changes in body composition and adiposity. Maturitas, 71(4), pp.320-325.
7. Poongothai, J.E.N.S., Gopenath, T.S. and Manonayaki, S.W.A.M.I.N.A.T.H.A.N., 2009. Genetics of human male infertility. Singapore Med J, 50(4), pp.336-347.
8. Smith, S., Pfeifer, S.M. and Collins, J.A., 2003. Diagnosis and management of female infertility. Jama, 290(13), pp.1767-1770.
9. Fenech, M., 1993. The cytokinesis-block micronucleus technique and its application to genotoxicity studies in human population, Eniron Health Perspect, vol. 101, no.3, pp.101-107.
10. Hsu, T.C., 1987. Genetic predisposition to cancer with special reference to mutagen sensitivity. In vitro cellular & developmental biology, 23(9), pp.591-603.
11. Moorhead PS, Nowell P C, Wellman W J et al, (1960). Chromasome preparation of leucocytes cultured from human peripheral blood Exp. Cell. Res; vol 20; Pp: 613-17.

LIST OF PUBLICATIONS

1. **Aneesh P**., AP Krishna., E Kayalvizhi., Drisya K., AnaswaraAA.,Dinesh Roy D., 2019. "Oxidative Stress and Genetic Instabilities in Polycystic Ovarian Syndrome". International Journal of advanced research (IJAR)., Int. J. Adv. Res. 7(9), 1336-1340.
2. ***Aneesh.P***, Sruthi K.C,Jisha.P, Jeena sharafudheen, Dr. A.P.Krishna.,2015 Prevalence of Thyroid Hormone Abnormalities among Pregnant Women International Journal of Scientific & Engineering Research, Volume 6, Issue 11, Nov-2015.

3. Jiju J.S, AswathySundaresh., Sheena Joe., **Aneesh. P.**, VijiJrishnan., Jeypal V., Dinesh Roy D., Vijayakumar T., 2014. "Correlation of Arterial Stiffness and the extent of Somatic DNA Damage in Cardiometabolic Syndrome". International Journal of Scientific and Engineering Research, IJSER/Volume 5, Issue 11, Nov 2014.
4. SumithaPrabhu., Sheena Joe., **Aneesh. P.**, Deepthi. T., ChandiniLal., Dinesh Roy D., 2016. "Risk Evaluation of Future Pregnancy among Patients with Recurrent

Abortion". Indain Journal of Applied Research. Volume 6/Issue 6/ June 2016/ISSN-2249-555X/IF:3.919/IC Value:74.50.

5. Jisha P., **Aneesh. P.**, RachanaRaveendran., Arun B., 2016. "A new light on TORCH infection among bad obstetric history cases". International Journal of Recent Trends in Science And Technology, ISSN 2277-2812 E-ISSN 2249-8109, Volume 17, Issue 3, 2016.
6. Minsa M., **Aneesh P.**, Leenamma Joseph., Lija L Raju, Krishna AP., Dinesh Roy D., 2016. "Biochemical and genetic studies on infertilesubjects and its risk for cardiovascular disease". Indian journal of applied research, Volume : 6 | Issue : 11, November 2016, ISSN - 2249-555X, IF : 3.919, IC Value : 74.50.
7. Lekshmi V.S., SumithaPrabhu P.S., **Aneesh P**., Arun William., Dinesh Roy D., 2020. "Oxidative stress and DNA damages in bad obstetric history". Paripex - Indian Journal of Research,Vol- 9, Issue- 3.
8. SumithaPrabhu P.S., JenivaBaruah., Komala P.T., Arun William., **Aneesh P**., Dinesh Roy D., 2020. "Role of cytogenetics in first trimester abortion using product of conception and extent of somatic DNA damages in maernal blood". Paripex - Indian Journal of Research,Vol- 9, Issue-3.
9. SumithaPrabhu P.S., Sharath Krishnan P.V., Komala P.T., Arun William., **Aneesh P**., Dinesh Roy D., 2020. "Cytogenetic and Molecular Cytogenetic Studies on Aborted Foetuses". Indian Journal of Applied Research, Vol- 10, Issue- 3.